PATENT COOPERATION TREATY

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# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 14 MAY 2004
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| Applicant's or agent's file reference<br>344677D20217 | **FOR FURTHER ACTION** | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| International application No.<br>PCT/IB 03/01789 | International filing date *(day/month/year)*<br>01.04.2003 | Priority date *(day/month/year)*<br>05.04.2002 |
| International Patent Classification (IPC) or both national classification and IPC<br>C12N1/36 | | |
| Applicant<br>INSTITUT PASTEUR et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 26.09.2003 | 13.05.2004 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized Officer<br>Weiland, S<br>Telephone No. +49 89 2399-7978 |

Form PCT/IPEA/409 (Cover Sheet) (January 2004)

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB 03/01789

## I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

**Description, Pages**

1-72 as originally filed

**Claims, Numbers**

1-59 as originally filed

**Drawings, Sheets**

1/16-16/16 as originally filed

**Sequence listing part of the description, pages:**

1-45, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   *(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-19, 21-24, 29-49 and 57-59 (all in part), 25, 27 and 50-56 (complete)

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-19, 21-24, 29-49 and 57-59 (all in part) and 25, 27 and 50-56 (complete)

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 24, 26, 28, 34, 41, 49 |
| | No: | Claims | 1-19, 21-23, 29-33, 35-40, 42-48 and 57-59 |
| Inventive step (IS) | Yes: | Claims | - |
| | No: | Claims | 1-19, 21-24, 26, 28-49 and 57-59 |
| Industrial applicability (IA) | Yes: | Claims | 1-19, 21-24, 26, 28-49 and 57-59 |
| | No: | Claims | - |

2. Citations and explanations

**INTERNATIONAL PRELIMINARY EXAMINATION REPORT**

International application No. PCT/IB 03/01789

**see separate sheet**

Re Item III

As the Applicant has not had an International Search Report drawn up on the other inventions, the present International Preliminary Search Report is based on the first invention for which the International Search Report has been established, i.e. for claims 26 and 28 (complete) and 1-19, 21-24, 29-49, 57-59 insofar as they refer to SEQ ID NO: 1 or fragments thereof. The Applicant should therefore limit the application to the invention searched and excise those parts of the application which relate to the other inventions.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

**1. General Remarks**

D1: MAHAIRAS G G ET AL: 'Molecular analysis of genetic differences between Mycobacterium bovis BCG and virulent M. bovis.' JOURNAL OF BACTERIOLOGY. UNITED STATES MAR 1996, vol. 178, no. 5, March 1996 (1996-03), pages 1274-1282

D2: WO 99 04005 A (STATENS SERUMINSTITUT ;ANDERSEN PETER (DK); RASMUSSEN PETER BIRK), 28 January 1999 (1999-01-28)

D3: BERTHET F X ET AL: 'A Mycobacterium tuberculosis operon encoding ESAT-6 and a novel low-molecular-mass culture filtrate protein (CFP-10).' MICROBIOLOGY (READING, ENGLAND) ENGLAND NOV 1998, vol. 144 ( Pt 11), November 1998 (1998-11), pages 3195-3203

D4: WO 9945119 A (FOLKERSON JOERGEN; STATENS SERUMINSTITUT (DK); JENSEN CARSTEN LOEV), 10 September 1999 (1999-09-10)

D5: ZHANG YE ET AL: 'The signalling pathway for BCG-induced interleukin-6 production in human bladder cancer cells.' BIOCHEMICAL PHARMACOLOGY, vol. 63, no. 2, 2002, pages 273-282,15 January, 2002

**3. Novelty (Art 33(2) PCT)**

3.1 Claims 1-19, 21-23, 29-33, 35-40, 42-48, 57-59 are not novel in view of D1. D1 already discloses M. bovis BCG bacteria reconstituted with the entire RD1 region (Acc. No. U34848, abstract and p. 1280, right column, 2nd paragraph), i.e. with part of the DNA fragment according to SEQ ID NO: 1. Moreover, D1 describes that the reintroduction of RD1 results in a protein expression profil almost identical to that of virulent M. bovis and M. tuberculosis. Likewise, D1 discloses plasmids (e.g. pGM910) comprising the entire RD1 region or fragments thereof (table 1 and p. 1280, right column, 2nd paragraph). D1 also proposes the use of the reconstituted M. bovis BCG for more effective and defined live attenuated tuberculosis vaccines (p. 1282, left column, 1st paragraph).

3.2 Additionally, claims 1-7, 15-19, 21-23, 29-33, 42-48, 57 and 58 are not novel over D2. D2 already describes immunogenic/ vaccine compositions against tuberculosis for intradermal administration comprising live attenuated bacteria, in particular M. bovis BCG, that have been reconstituted with sequences of the RD1 region, i.e. "part of" the RD1 region, more specifically with sequences encoding CFP-10 (i.e. LHP) and ESAT-6 (p. 5, last paragraph; p.6, line 20 to p. 7, line 12; p. 17, 3rd paragraph; p. 44, last paragraph to p. 46, 2nd paragraph; claims 12, 49 and 53 a). Likewise, D2 also discloses plasmids comprising said sequences (e.g. pIPX26, pIPX61, pIPX15 and pIPX16) and promotors allowing the expression of peptides encoded by sequences of the RD1 region in Mycobacteria as well as M. bovis BCG bacteria transformed with these plasmids (see fig. 1 and 2 and descriptions thereof, claims 6 to 8). D2 also considers vaccine compositions comprising live M. bovis BCG strains transformed with sequences encoding CFP-10 and ESAT-6 as well as antigenic portions of CFP-10 (Claims 27 and 48-53 d).

3.3 Additionally, claims 1-19, 21-23, 29-33, 35-40, 42-45, 57-59 are not novel over D3. D3 already discloses a M. bovis BCG strain transformed with nucleic acids comprising RD1 sequences, in particular the GFP-10 (i.e. LHP) and the ESAT-6 gene sequence, the coding sequences being in frame with their natural promotor (p. 3198, right column, last paragraph to p. 3199, left column, line 3). The constructs were able to induce expression of the coding sequences in Mycobacterium (p. 3199, left column, lines 11 and 12). Correspondingly, plasmids comprising sequences of RD1 for the transformation of M. bovis BCG are disclosed in D3 (pIPX15, 16 and 61, see Table 1 and Fig. 1).

3.4 Finally, claims 1-7, 15-19, 21-23, 29-33, 42-48, 57 are not novel in view of D4.

## 4. Inventive Step (Art 33(3)PCT)

4.1 The subject-matter of claims 24, 26, 28 and 41 of the present application differs from the subject-matter of D1, which is considered to be the closest prior art for these claims, in that a specific cosmid (RD1-2F9) with a specific accession number (I-2831) or its insert with a specific sequence (according SEQ ID NO: 1) is referred to. The insert of RD1-2F9, corresponding to SEQ ID NO: 1, differs in length from the fragment (Acc. No. U34848) for transformation of M. bovis BCG as disclosed in D1 (ca. 32 kb instead of 17499 nt). However, as the technical effect of this difference is not apparent, the subject-matter of claims 24, 26, 28 and 41 does not involve an inventive step.

4.2 D5 which is considered to represent the closest prior art for claim 49 discloses the activation of the local, probably T lymphocyte-dependent, immune response by means of *M. bovis BCG* as standard treatment of superficial bladder cancer and also the fact that only 60-70% of the patients respond to this therapy (abstract and p. 273, left column, 1st and 2nd paragraph). The difference between D5 and the present invention is that claim 49 refers to the use of a M. bovis BCG strain reconstituted with the RD1 region as adjuvant/immunomodulator. The effect of the transformation of M. bovis BCG with the deleted region is a longer persistence and higher virulence of M. bovis BCG in the infected organisms conferring a stronger induction of the immune response. Thus, the problem solved by the present invention appears to be the provision of a M. bovis BCG strain that is more efficient in stimulating the immune response in the treatment of superficial bladder cancer. However, D2 discloses recombinant M. bovis strains containing RD1 sequences and expressing CFP-10 and ESAT-6 considered to act synergistically in the stimulation of the immune response for medical use (e.g. p. 17, 2nd paragraph). A person skilled in the art looking for a more efficient treatment of superficial bladder cancer with M. bovis BCG would have combined the two documents and would have arrived at the subject-matter claimed by claim 49.

4.3 Claim 34 does not involve an inventive step neither. Contrastingly to the cited prior art, the claim refers to mutated genes within the RD1 region. However, as no gene or mutation is specified, the technical effect of this difference is not apparent.

Thus, claim 34 does not seem to solve a technical problem nor to comprise an inventive step.

4.4 Therefore, the subject-matter of claims 24, 26, 28, 34, 41 and 49 does not meet the criteria of Art 33(3) PCT.

## 5. Further Remarks

5.1 With regard to the claims 19, 22 and 23 the attention of the Applicant is drawn to the fact, that said claims do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempt to define the subject-matter in terms of the result to be achieved which merely amounts to a statement of the underlying problem. In fact, said claims lack the technical features necessary for achieving this result.